Correspondence

Spinning nuclei in the brain of the zebrafish embryo

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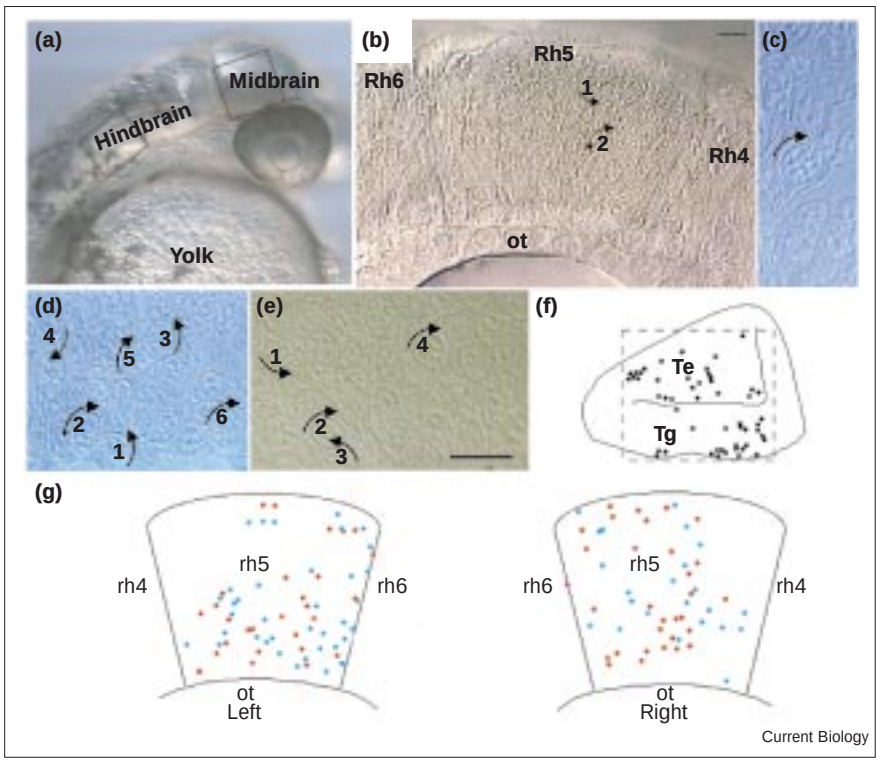
I wish to report here a striking phenomenon that arises in the developing brain of the zebrafish embryo. High-resolution differential interference contrast (DIC) time-lapse video microscopy in the live embryo reveals that throughout the second day of embryogenesis, the nuclei of some of the still-undifferentiated brain cells undergo a sustained spinning behaviour, usually around a constant axis specific to each nucleus. Figure 1 shows several nuclei in the brain that were spinning around an axis perpendicular to the focal plane (see Supplementary material). The mean spin rate is about 10 rotations per hour, but some nuclei were spinning at up to one rotation per minute over a 30-minute period, with a peak velocity of two rotations per minute (Figure 1e).

Spinning can occur around any axis (but see below) and can be continuous or saltatory. Most nuclei spin in the same direction for the whole duration of the video recording (up to 2 hours); cases of reversal, or change in spinning axis, are shown in Figure 1b,d. In cell fields in which several nuclei were spinning simultaneously in the same vicinity, no obvious correlation could be discerned between their individual axes of rotation. The spinning behaviour also seems to be unlinked to mitosis, which can be readily observed in the brain.

In the hindbrain, spinning nuclei were first observed at about 24 hours post-fertilization (hpf, in standard developmental time [1]), and from then up to the latest stage examined (42 hpf). In the midbrain, spinning nuclei were first observed by 30 hpf, both in the optic tectum and in the tegmentum (Figure 1f), although in the latter, spin rates as

Figure 1



Spinning nuclei in the developing hindbrain and midbrain of the live zebrafish embryo, revealed by high-resolution DIC time-lapse video microscopy. The nuclei, but not the cell boundaries, of brain neuroepithelial cells are clearly visible. These nuclei may spin around any axis, but rotations can be counted only when the axis of rotation is orthogonal to the focal plane, that is, when nuclei are rotating clockwise or anticlockwise relative to the observer. All embryos were observed in lateral views. **(a)** Lateral view of the head of a live zebrafish embryo at 30 h post-fertilization (hpf). Boxes indicate the two brain areas surveyed at higher magnification in the subsequent panels. **(b–e)** Nuclei spinning during the recording session are identified by a number and an arrow showing their direction of rotation. (b) View of the right side of the hindbrain centred on rhombomere 5 (Rh5) dorsal to the otic vesicle (ot), at 26 hpf. Nucleus 1 performed nine rotations in 25 min, and nucleus 2 first rotated seven times in an anticlockwise direction over 19 min, and then eight times clockwise in 28 min; this nucleus is shown at higher magnification in (c) as it continued to spin in a clockwise direction. (d) Hindbrain (left side), at the same location as in (b), but at 29 hpf. Nucleus 2 performed 11 clockwise rotations in 12 min, 2.5 anticlockwise rotations in 7 min, then 7.5 clockwise rotations in 24 min. Nucleus 1 rotated six times in 23 min, nucleus 3 rotated three times in 12 min, and nucleus 4 rotated twice in 7 min. Nuclei 5 and 6 both made two clockwise rotations in 11 min; subsequently their spin axes changed to a direction non-orthogonal to the focal plane. (e) Midbrain tegmentum (right side), at 37 hpf. Nuclei 1 and 2 rotated 23 times in 24 min and 10 times in 8.5 min, respectively, both with a peak velocity of two rotations per min. Nuclei 3 and 4 rotated 2.5 and 3 times in 13 and 21 min, respectively. **(f)** Positions of all spinning nuclei observed in the left midbrain between 30 and 40 hpf, from 29 recording sessions (28.5 h overall) made at different focal planes in 12 embryos. The area surveyed is boxed. Te, tectum; Tg, tegmentum. **(g)** Positions and sense of rotation of those nuclei spinning in the focal plane in the left and right parts of rhombomere 5 dorsal to the otic vesicle. Left view: 14 recording sessions (13 h overall), in seven embryos; right view: 14 sessions (10.3 h overall), in five embryos. Blue and red dots indicate clockwise and anticlockwise rotations respectively. Scale bars represent 10 μm; (c–e) are at the same magnification.

fast as those found in the hindbrain only occurred after 35 hpf (Figure 1e). In the hindbrain and the midbrain tegmentum, medio-laterally oriented spinning axes (that is, rotations in the observation plane) were predominant, whereas in the optic tectum, most nuclei did not spin around a medio-lateral axis — a difference that might be related to the different orientation of the neuroepithelium in that part of the brain. Because it is less accessible, the forebrain was not investigated. Interestingly, the neuroretina, although it originates from the brain neuroepithelium, did not have spinning nuclei at any stage of its development.

A more extensive survey of the distribution and direction of nuclear rotations occurring in the observation plane was carried out in a well-defined compartment of the hindbrain, rhombomere 5, the limits of which can be fairly well discerned in the living embryo. Both clockwise and anticlockwise rotations were found throughout the rhombomere, with a somewhat reduced frequency anteriorly, both on the left and the right side of the rhombomere (Figure 1g).

In the hindbrain or midbrain of the day-2 embryo, only a small minority of the cells of the neuroepithelium have already differentiated into neurons [1]. Although the larger, easily recognizable nuclei of these young neurons occasionally undergo partial or even complete rotations during a 1 hour recording session, this is by no means comparable to the high spin rates displayed by undifferentiated cells of the neuroepithelium.

Only one report, by Pomerat in 1953 [2], concerning tissue cultures of adult human nasal mucosa, documented nuclei spinning at rates comparable to, although lower than, those reported here in the zebrafish embryo. Some later papers reported nuclear rotations in tissue cultures of neurons and in some cell lines, but at much lower rates (up to one rotation per hour, at most) [3–7].

The very high spinning rates found here in the zebrafish embryo occur only in the still undifferentiated brain neuroepithelium, and in a well defined developmental sequence — first in the hindbrain and subsequently in the midbrain. This observation raises at least three lines of questioning: how are the spinning nuclei anchored to the surrounding cell structures; what is the motive force that can make them spin so fast; and what is the possible functional significance of this phenomenon for the neuroepithelial cells involved?

Supplementary material

Supplementary material including time-lapse video recordings of the spinning nuclei shown in Figure 1 is available at http://current-biology.com/supmat/supmatin.htm.

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Sensitivity of the yellow variant of green fluorescent protein to halides and nitrate

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Several groups [1,2] have recently reported the use of variants of green fluorescent protein (GFP) as a visual reporter of intracellular pH in living cells, and in principle such techniques could also report spatial variations of pH in real time using a fluorescence microscope. It has been demonstrated that the chromophore of GFP, which is derived by autocatalytic cyclization and oxidation of the primary sequence -Ser(Thr)65-Tyr66-Gly67-, can be titrated at the Tyr66 phenolate oxygen [3], and that the ionization constant, pK_a, of this group depends on the surrounding environment. We have constructed variants with pK_a values ranging from 6–8, so that pH in the range of 5–9 can be reliably reported in the cell [4].

Recently, we discovered that an engineered variant of GFP, yellow fluorescent protein (YFP), which contains the substitution Thr203 → Tyr, plus additional mutations to improve brightness in living cells (S65G/V68L/S72A in the single-letter amino-acid notation) [5], has a pK_a which is dependent on the concentration of halide or nitrate ions, and hence, both the absorption and emission spectra vary. The ionization constant does not depend on the concentration of metal cations or other anions, such as mono- and dibasic phosphate, sulfate, carbonate, acetate, gluconate, or formate. We have constructed variants on the YFP background in which the pK_a of the chromophore ranges from 5.2–7.0 (YFP), 6.7–7.7 (YFP-H148Q) and 7.5–8.2 (YFP-H148G), over a chloride concentration range of